The Growth of the Oocyte in Antedon: a Morphological Study in the Cell-Metabolism.

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(Abstract.)

In this paper I have endeavoured to interpret in terms of the cell-metabolism the structural changes exhibited by the growing ovarian egg of *Antedon bifida*, Penant. The following conclusions are based entirely on the study of this single type. It will be my endeavour in subsequent papers to ascertain how far these conclusions are applicable to the eggs of other types and to the cell in general.

The Yolk Nucleus.—Throughout the growth of the oocyte the nucleolus intermittently discharges groups of deeply basophile spherules into the cytoplasm. In the young oocyte these "nucleolar spherules" remain unchanged, slowly accumulating in the cytoplasm, where they form small groups near the germinal vesicle. In slightly older oocytes the increased fluidity of the cytoplasm which results from the progressive accumulation of metaplastic material in preparation for yolk formation, causes the discharged nucleolar substance to lose its spherular form, and to diffuse on to the neighbouring cytoplasm. The more deeply staining area of the cytoplasm to which this diffusion gives rise is the yolk nucleus. As the cytoplasm becomes still more fluid, the yolk nucleus assumes the regular form of a concavo-convex lens embracing the spherical germinal vesicle.

During yolk formation the yolk nucleus passes to the periphery of the egg, a migration also shared by the "peripheral spherules." The actual increase in size which the peripherally placed yolk nucleus shows during the earlier stages of yolk formation is speedily masked by the great superficial extension and thinning out on the surface of the egg which this structure undergoes during yolk formation, and which is the necessary result of the great increase in bulk and surface area accompanying this process.

As yolk formation progresses the yolk nucleus gradually loses its staining capacity, and, before this process is completed, has entirely disappeared from view. At no time, however, does the yolk nucleus show any indication of a granular disintegration, nor does it show any spatial relation to yolk

formation. Yolk formation takes place in this area of the cytoplasm exactly as elsewhere.

The clear area often accompanying the yolk nucleus, usually on its outer surface, is an artifact, and results from the inability of the acid fixing reagent to coagulate the metaplastic substances with which the cytoplasm, and especially the yolk nuclear area of the cytoplasm, is loaded.

The yolk nucleus, therefore, is simply a region of the cytoplasm on to which waste material discharged from the nucleolus has diffused. The period at which this diffusion occurs, as also all subsequent changes in the form and position of the yolk nucleus, are determined by the progressive change in the physical consistency of the cytoplasm which precedes and accompanies yolk formation. Thus the yolk nucleus, like the nucleolar material discharged subsequent to its formation, takes no part in the cell-processes.

The Nucleolus.—The spherical form of the nucleolus, a form in which minimal surface area is associated with maximal bulk, stands in striking contrast to the subdivided condition of the chromatin, and indicates the absence of any reaction between the caryolymph and the nucleolar substance at the surface of the nucleolus; the latter structure passively growing by the deposition of material on its surface from solution in the caryolymph.

This nucleolar material consists of two substances: the one acidophile and extending throughout the nucleolus, the other deeply basophile and borne by the acidophile ground substance, to which its presence imparts a considerably firmer consistency.

Nucleolar activity, which proceeds from within outwards, results in the breakdown of the basophile constituent; the products of this process forming an irregular zone between the firm, as yet unaltered, basophile cortical region and the acidophile internal region of the nucleolus. When the internal pressure reaches a certain point the cortical layer is ruptured, and the accumulated products are discharged into the caryolymph, partly as deeply basophile spherules, partly as a clear fluid. The spherular form of the discharged material is determined by the fluid character of the surrounding caryolymph, and when the accumulated products of nucleolar activity happen to lie within a vacuole in the nucleolus, then they here also possess a spherical form. There is no indication that the acidophile ground substance of the nucleolus is itself discharged.

During the growth of the oocyte the amount of the deeply basophile nucleolar substance varies greatly, but the growth of the nucleolus nevertheless shows a strict correlation with that of the egg, and is due to the steady accumulation of the acidophile ground substance.

On the completion of yolk formation all indication of nucleolar activity abruptly ceases. The supply of the basophile constituent of the nucleolus also ceases, but the acidophile constituent continues to be deposited on to the nucleolar surface, where, no longer being incorporated by the now inactive nucleolus, it forms lens-shaped accumulations.

The selective action shown by the nucleolar material, together with the strict correlation the growth of this structure shows to that of the egg, excludes the possibility of this substance being derived directly from a source outside the cell. The fact that the ground substance continues to be deposited on to the nucleolus after the completion of all cytoplasmic activity, together with the close relation this structure shows to the chromatin in earlier stages, points to the latter as its source. The failure of the supply of the basophile constituent on the completion of yolk formation indicates its origin from the cytoplasm.

The steady growth of the nucleolus is not due to the accumulation of waste material, but to an increased production of the "ground substance" by the chromatin in order to cope with the ever-increasing production of waste material by the cytoplasm of the growing egg. From the nucleolus this waste material, now presumably inert and harmless, is discharged into the cytoplasm, where it slowly dissolves away.

The Chromatin.—With the increase in metabolism which accompanies the growth of the oocyte there is a corresponding finer subdivision, and more equal distribution of the chromatin. This endeavour to increase the facilities for reaction with the surrounding caryolymph indicates that the chromatin obtains its food material by active incorporation instead of passively by deposition. The absence of any reciprocal action on the part of the food material of the chromatin indicates for this substance a source outside the cell. The chromatin is, therefore, the cell-structure with which the raw food material supplied by the parent organism first comes into relation.

During the gradual accumulation of metaplastic material in the cytoplasm which marks the period preceding yolk formation, the chromatin is sparsely scattered and relatively faintly stained. With commencing yolk formation the staining capacity, and hence also the quantity visible, of the chromatin rapidly increases, and at the same time basophile droplets appear on the chromatin threads and increase in number and size during yolk formation.

The elaborated food material is passed on from the chromatin, in part to the nucleolus, but mainly to the cytoplasm. During the period preceding yolk formation, and which is marked by the progressive accumulation of metaplastic material in preparation for this process, the avidity with which the cytoplasm takes up the products of chromatin activity causes the latter

structure to appear faintly stained. With the commencement of yolk formation this avidity is greatly reduced and the products of chromatin activity, now being produced in excess of the requirements of the cytoplasm, accumulate on the chromatin threads, causing the increase in the staining capacity of the latter and the formation of the basophile droplets.

The Cytoplasm.—Throughout the whole growth period of the oocyte there is a gradual accumulation in the cytoplasm of metaplastic material in preparation for yolk formation. This accumulation is accompanied by an increase in the basophile staining capacity, and fluid consistency, of the cytoplasm. The process of yolk formation, that is the actual appearance of the definitive yolk spherules, is unaccompanied by increased nuclear or nucleolar activity, and consists simply in the rapid and automatic conversion of the accumulated material into a form sufficiently stable to survive the period of quiescence which succeeds the completion of the egg's growth.

The "Germinal Vesicle."—Form of the Nucleus.—The oocyte constitutes an osmotic system, of which the cell wall forms an outer, the nuclear membrane an inner, semipermeable membrane. The accumulation in the cytoplasm of soluble substances in preparation for yolk formation causes an increase in the osmotic intensity at the outer membrane, and a corresponding decrease of that at the inner. The loss of turgescence which thus results permits of the assumption of an irregular form during fixation.

Size of the nucleus and subdivision of the chromatin.—The large size of the germinal vesicle, as also the fine subdivision of the chromatin, are due to the limitations which the care of the specific qualities imposes on the adaptive capacity of the chromatin; both these conditions being merely the expression of a more economic use of this material. The subdivision of the chromatin increases the area of contact with the caryolymph, and the increased area of the nuclear membrane through which diffusion takes place permits of a greater concentration of nutritive material within the nucleus. Up to the expansion due to the formation of the definitive yolk spherules, therefore, the size of the germinal vesicle shows a close relation to that of the egg, and is determined when equilibrium is established between the rate of diffusion of nutritive substances through the nuclear membrane on the one hand, and the requirements of the chromatin on the other.